

## Response to comment on "The interaction of cells and bacteria with surfaces structures at the nanoscale"

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# Response to comment on « The interaction of cells and bacteria with surfaces structures at the nanoscale."

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<sup>1</sup>Institut de Science des Matériaux de Mulhouse (IS2M), CNRS LRC7228, Université de Haute-Alsace, Mulhouse, France <sup>2</sup>Abteilung Schutz und Physiologie, EMPA, St. Gallen, Switzerland <sup>3</sup>Centre Suisse d'Électronique et Microtechnique (CSEM), Neuchâtel, Switzerland

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\*Karine Anselme Mailing address: IS2M, 15 rue Jean Starcky, BP2488, 68057 Mulhouse cedex, France Phone number: +33 389608766 Fax number: +33 389608799 Email address: <u>karine.anselme@uha.fr</u>

<sup>§</sup>Lydie Ploux Mailing address: IS2M, 15 rue Jean Starcky, BP2488, 68057 Mulhouse cedex, France Phone number: +33 389608798 Fax number: +33 389608799 Email address: <u>lydie.ploux@uha.fr</u> In agreement with Dr. Schilardi and collaborators, the authors are also convinced that bacteria are far from simple colloidal particles and that they are able to sense topographical features of the surface and to react accordingly, which nevertheless has not been strictly demonstrated so far. However, it cannot be expected that bacteria are completely protected from processes affecting colloidal particles, especially from dewetting processes [1]. The author's conviction is that the experiments conducted by Dr. Schilardi and coll. in their recent article [2] were affected by dewetting-related experimental artifacts. In particular, bacteria localization may have been modified by air or nitrogen drying, even in a relative humidity greater than 70% or when the sample was kept in air during a short time before immersing it again (conditions used by Schilardi and coll. for AFM imaging and for the washing procedure prior to epifluorescence imaging, respectively). A dewetting process occurs whatever the humidity of the surrounding air environment [3,4]. It is due to the liquid evaporation, leads to meniscus formation, convective flow, rupturing, dewetting, and capillary-bridge formation. As soon as environmental conditions allow evaporation of the colloidal suspension, colloidal particles are driven by the threephase contact lines, which will lead to their organization [5,6]. Bacteria are unlikely to be able to resist to these driving forces. Our review (figure 9) [7] displays an example of such colloidal-like behavior of bacteria. Another example, leading also to bacteria organization and alignment with grooves, is given in Figure 1. Bacteria orientated much more with grooves when colonized substrate was dried before to be immersed again in liquid. Despite the longer time the substrate stayed in air in our experiment compared to those of Dr. Schilardi and coll., this demonstrates that dewetting processes can affect bacteria organization. Finally, it can be asserted that experimental dewetting-related artifacts play a role in the results of studies addressing bacteria organization to surface, as soon as an interface between the substrate and air is created before observation. Therefore, the conclusion made by Dr. Schilardi and her group in the article [2] should be rather considered as a hypothesis that still needs to be demonstrated.

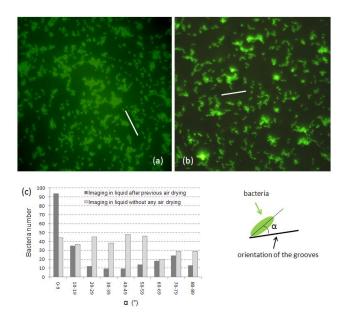


Figure 1: Adherent *Escherichia coli* K12 on microstructured substrates (see [8] for more details about the substrates) observed in immersion using epifluorescence microscope. (a) Substrates were kept in air during about 1h before being immersed again in NaCl 9g/L solution. (b) Substrates were always kept immersed. (e) Orientation of the bacteria present on (a) and (b) micrographs in comparison with the grooves direction (indicated on the micrographs by the white bars).

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